DAU/GRC/NIA/NIH
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GEL ANALYSIS OF PCR PRODUCTS FOR ARRAY PRODUCTION

Purpose: to evaluate relative size and intensity of bands

Materials Needed:

- DNA ladder 250bp (Gibco/Life Tech, cat# 10596013)
- Agarose
- Power Supply 300V (Owl Sep. Systems, cat# OSP300)
- Gel combs, 50 tooth (Owl Sep. Systems, cat# D3-MT2D)
- Gasketed UVT gel tray (Owl Sep. Systems, cat# D3-UVT-14)
- Centipede wide format gel system (Owl Sep. Systems, cat# D3-14)
- Casting chamber (Owl Sep. Systems, cat# D3-CST-14)
- 12.5 ul Matrix pipettor (Apogent Discoveries, cat# 2019)
- 12.5 ul pipet tips from PCR protocol
- P20 pipettor
- Universal tips, 30 or 200 ul
- 50X TAE
- ethidium bromide

Preparation:

1. Using 10X TAE buffer stock [0.4 M Tris-base (96.88 g), 0.05M Sodium acetate (13.6 g sodium acetate – 3 H_20), 0.01 M EDTA (7.44 g) in 2 liters water, pH to 7.6 with 12N HCl], prepare enough 1X TAE to make the gels and to fill the gel boxes. Each gel box requires at least one liter of fluid, and each gel requires 200ml.
2. To make the gels, measure out 4 grams of agarose and 200ml 1X TAE for each gel. In a microwave-safe liter glass bottle, mix the powder and liquid and heat for 5-6 minutes in a microwave. NOTE: Do not attempt to make more than two gels per liter bottle, as the gel will boil over.
3. Cool the hot bottle in a bucket of hot water, until the bottle is cool enough to be handled. Do not pour the gel when it is very hot, as this can warp the gel trays. NOTE: Be very careful when handling the hot bottle, as the gel mixture is volatile and will blow out of the bottle if shaken too hard.
4. Fit the gasketed gel tray into the casting chamber, making sure the gaskets are tight and the tray level.
5. When the agarose/TAE mixture is cool, add 3-5 ul ethidium bromide per gel, swirl, and pour 200 ml into the tray.
6. Remove any air bubbles with a paper towel.
7. Fit four gel combs into the slots provided. There are six slots. So that each row of wells is evenly spaced, from the top, fit the combs into slots one, two, four and six.

8. Let the gel set before using.

Procedure:

1. Remove plates from thermalcycler just prior to use. [Seal cover and store at -20°C after sample removal.]

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2. Withdraw 2 ul (using tips saved from when you loaded the plasmid into the PCR) from each well of the PCR plate working one row at a time. Program the multichannel pipetter to fill with 2 ul and dispense 2 ul.

3. Load samples in gel. Skip first and last lanes for markers. Thus, begin loading the A1 sample in the 2nd gel well from the first comb. Multipipettor tip spacing is twice as wide as the comb well spacing, thus you load each row (of 96 well plate) into every other well, when loading with the multipipettor. The second (B) row of the 96 well plate will be loaded in the alternate wells, between the samples of the first (A) row. This second loading (samples B1-B12) begins at gel well #3. Similarly, when loading the C and D rows, the C1 sample will be loaded into the 26th well, and the D1 sample will be loaded into the 27th well. Resulting lanes will contain:

Marker, A1, B1, A2, B2, A3,…… A11, B11, A12, B12, C1, D1, C2, D2,…….C11, D 11, C12, D12.

Likewise the E, F, G and H set of samples will go into the next row of gel wells (made using a second comb). Their final order will read:

E1, F1, E2, F2, E3,……. E11, F11, E12, F12;G1, H1, G2, H2,…….G11, H11, G12, H12.

Thus, each 96 well plate requires two complete gel combs, and each gel (with 4 combs) will hold 2 x 96 well plates worth of samples (with markers for each lane).

4. Load 10 ul of DNA marker in each outside lane. Run gels at 115 volts for 39 minutes. Remove gel, read on UV lightbox, take photo, and save image. (Fig 1)

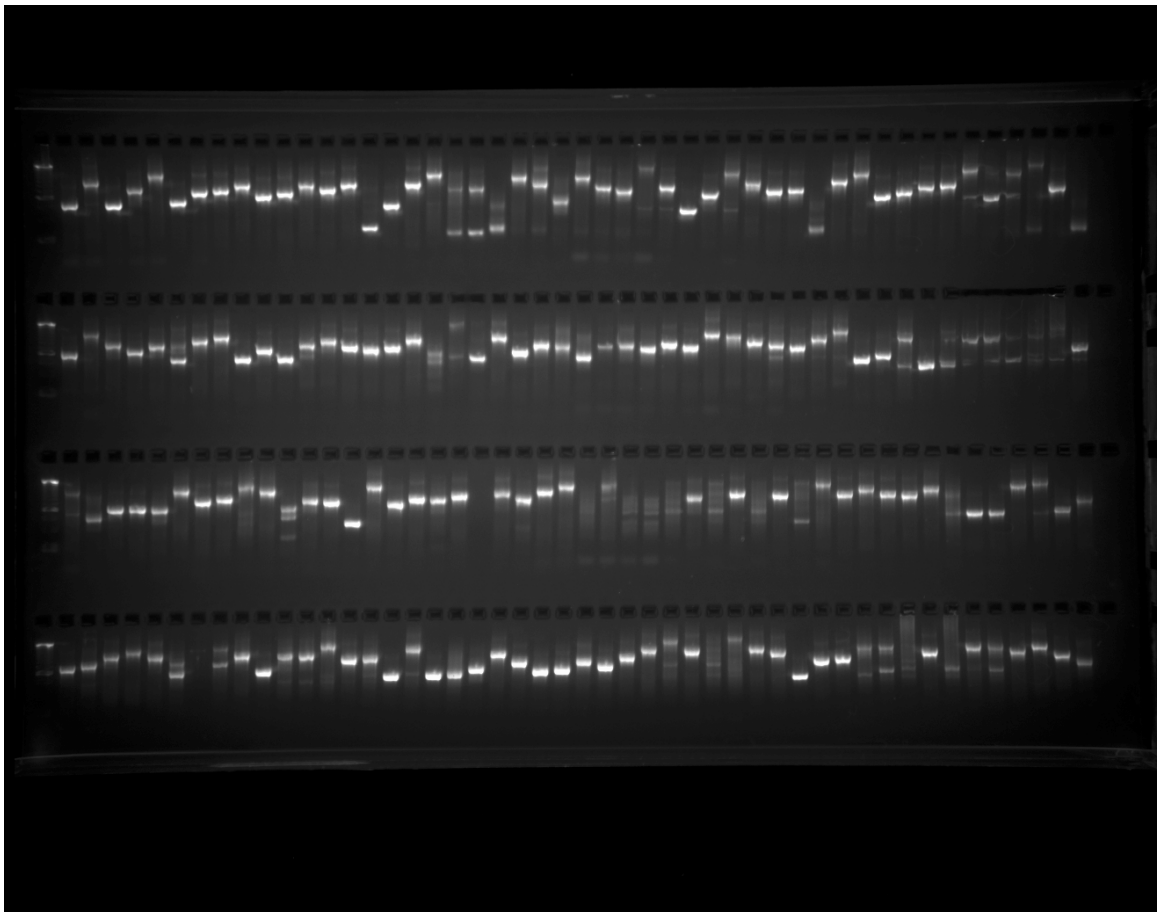

Figure One: Example Gel Photo Using The Above Protocol

Comments:

1. The PCR product that is run in the gels is very light colored and a volume of 2ul cannot be easily seen. In addition to using the above protocol of loading every other well, it is helpful to immerse the gel in the gel box so that the wells fill with buffer, then remove the gel and place it on a plastic tray to load.

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For frequently asked questions go to the following address:
http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm

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